Remarks

Receipt is acknowledged of the Office Action dated July 3, 2002 in the above-captioned matter. Reconsideration of the application and a three month extension of the time provided for a response is respectfully requested. A check for $1338 in payment of the extension fee ($930) and the fee for the new claims ($408), is enclosed. With respect to the new claims, 18 claims have been added, including 2 independent claims (for a total of 4 independent claims in the application), resulting in a fee of $408 ($324 for the total number of new claims, and $84 for the fourth independent claim). The Commissioner is hereby authorized to charge Deposit Account No. 50-1604 for any additional fees that may be deemed necessary in conjunction with the present application, and is also authorized to credit any overpayments thereto.

In the Office Action, the Examiner rejected the application under 35 U.S.C. §112 for use of the term "corresponding feature" in Claims 1 and 19. The clause referred to the spot on the microarray having probe sequence. However, these clauses have been deleted in the above amended claims, since the language is not essential, and since the claim is clear without it. No narrowing of the claim has been effected nor is intended, rather a slight broadening has been effected. In addition, various uses of the term "containing" have been amended to use the term "comprising" instead, and likewise other unnecessary has been deleted to broaden the claims as well.

In the Office Action, the pending claims were rejected under 35 U.S.C. §103(a) based primarily on Skouv (U.S. Patent No. 6,303,315) and Chee (U.S. Patent No. 6,355,431), with additional rejections based on Shimuzu (U.S. Patent No. 5,391,653) and English (U.S. Patent No. 6,077,824). Reconsideration of the rejections is respectfully requested.

As is currently well known in the art, nucleic acid samples in the form of RNA are often

extracted from cells for testing in assays for a wide range of different purposes. RNA, however, is relatively unstable and decomposes rapidly and easily. As a result, a more stable and resistant form of nucleic acid known as complementary DNA (cDNA) is typically used. The RNA in the sample is reverse transcribed to produce the more stable cDNA, which is then studied in the assay.

The present application is directed to a method for preparing a nucleic acid sequence sample for use in a microarray assay in a manner which significantly reduces the time and effort traditionally required for an assay procedure. In accordance with the present invention, a method is provided which allows hybridization of the RNA extracted from a sample to be directly hybridized to probe nucleic acid, without reverse transcription of the RNA to cDNA. By eliminating the need for reverse transcription, a significant amount of time is eliminated. Moreover, since this method is used in conjunction with a microarray, thousands of "test-tube" like experiments can be conducted simultaneously.

None of the cited references teach or suggest this method, whether individually or in combination. Skouv, for example does not teach the method of assaying target nucleic acid by applying it to probe nucleic acid affixed to a microarray. Moreover, even though Chee discusses the use of arrays, it would not be obvious to combine the two references. Skouv teaches a method requiring the synthesis of a probe reagent in the form of a modified nucleic acid known as a locked nucleic acid (LNA). This LNA probe reagent must be synthesized for each target nucleic acid to be studied. *See e.g.*, col. 3 lines 25 - 30 ("Such methods include lysing the cells ... contacting the lysate under hybridisation conditions with a locked nucleic acid (LNA) having a nucleotide sequence substantially complementary to a nucleotide sequence suspected to be present in the cells, and determining the extent of hybridisation"); col. 7 lines 44-48 ("Sequences suitable for capturing or signalling nucleic acids for use in hybridisation assays can be obtained from the entire sequence or portions thereof of an organism's genome, from

messenger RNA, or from cDNA ..."); col. 7 lines 57-59 ("Once the appropriate sequences are determined, LNA probes are preferably chemically synthesized using commercially available methods and equipment as described in the art").[1]

If the teachings of Skouv were to be combined with the teachings of Chee for use in a microarray assay, the assay would be unmanageable. In a microarray experiment, the user does not use one or two probe nucleic acids, but hundreds, or thousands, or tens of thousands, or more. A microarray is a substrate having a grid of thousands of tiny spots ("features") of probe nucleic acid, with each spot of probe having copies of a short sequence of nucleotides. A computer keeps track of the specific nucleotide sequence which is located at each spot. Therefore, by using the microarray, one can study extremely large numbers of different spots virtually simultaneously.

Were Skouv to be combined with Chee for use in a microarray experiment as claimed, an LNA probe would have to be synthesized for each spot on the array. In other words, thousands of different LNA reagents would have to be synthesized – one for each of the thousands of spots of probe located on the array. This would be an extremely burdensome, time consuming, and expensive process. As a result, it would not be at all obvious to one of ordinary skill in the art to combine the teachings of the references. Combination of the references would not ease the user's burden, it would increase and multiply it.

Since the methods of Skouv are directed to use with LNA only, one of ordinary skill in the art would not be led to combine the teachings of the Skouv and Chee references. In contrast, the present

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[1] While Skouv occasionally uses the term "capturing nucleic acid" (see e.g. col. 7 lines 1-5), he is referring to the probe immobilized to the surface of the assay. See e.g. col. 7 lines 1-2 and col. 6 lines 6-8 (the latter referring to the "capturing LNA probe". It should be noted that this is not the same as the "capture reagent" of the present application, which is the labelled nucleic acid that delivers signal.

method is possible in conjunction with the large number of features present on microarrays, without requiring the synthesis of large numbers of special LNA reagents for each of those features, since the present invention can be used with probes made of traditional nucleic acids (e.g. DNA or RNA). Accordingly, the presently claimed methods using microarrays are believed to be fully patentable over Skouv and Chee, both individually and in combination.

In addition to the previously pending claims, new dependent Claims 39-42 have been added. These claims are patentable for all of the reasons set forth above with respect to Claims 1 and 19. Moreover, these new claims further recite the embodiments in which the probe nucleotide sequence is made of traditional nucleotides of DNA (including cDNA) or RNA.

The Skouv reference does not teach the use of probes made of traditional nucleotides. Rather Skouv teaches away from the use of traditional DNA or RNA nucleotide probes. The method disclosed in Skouv is designed for use with LNA only – i.e. only with chemically modified nucleotides. *See e.g.*, col 2 lines 37-41 ("A crucial component in the invention is LNA which is a novel class of DNA analogues ...). As a result, the combination of the Skouv and Chee references teaches away from the use of probes made of traditional nucleotides as recited in Claims 39-42.

Claims 1 and 19 have also been broadened with respect to the second component, such that it is not limited to a single molecule, but include any one or more molecules which are associated together, so as to both bind the RNA reagent and provide a hybridization pattern. Thus, the second component can be a single molecule, a complex of two or more molecules, or so forth. As an example of a single molecule, a molecule can be provided of dendrimer having a capture sequence on one arm, and a label on another arm. As one example of a complex, the second component can include a first molecule comprising the capture sequence, and a second molecule which both binds the first molecule and which

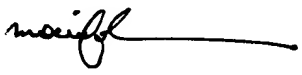
also includes a detectable label.

New independent Claims 47 and 52 (and claims dependent thereon) have been added hereto as well. Claims 47 and 52 include the limitation that the probe nucleotides are DNA and RNA. In various other respects, these claims have been broadened with respect to prior Claims 1 and 19. Yet, although broadened, Claims 47-56 all recite features of the invention patentable over both Skouv and Chee and the remaining references, as previously discussed.

Accordingly, based on the above, it is respectfully submitted that the claims of the application are currently all in fully allowable form. Favorable action on the application and allowance of the pending claims is respectfully requested and believed fully warranted.

Dated: January 3, 2003

Respectfully submitted,



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Getts & Kadushin	Patent Application
Serial No.:	09/908,950	
Filing Date:	July 19, 2001	
Examiner:	Shar Hashemi	
Art Unit:	1637	
For:	Methods for detecting and assaying nucleic acid sequences	
Attorney Docket No.:	4081.006	

Marked-Up Amended Claims

For the Examiner's reference and pursuant to 37 C.F.R. 1.121, a marked-up version of the amended claims is provided as follows:

1. (Once Amended) A method for determining the presence of a specific nucleotide sequence in an RNA reagent of a target sample, said method comprising the steps of:

a) incubating a mixture comprising:

(i) a first component including an RNA reagent extracted [directly] from a target sample, said RNA reagent having a target nucleotide sequence and a capture sequence, and

(ii) a second component comprising a capture reagent [having at least one first arm containing] <u>comprising</u> a label capable of emitting a detectable signal and [at least one second arm having] <u>comprising</u> a nucleotide sequence complementary to the capture sequence of the RNA reagent of the first component,

at a first temperature [and for a time sufficient] to induce the capture sequence of the RNA reagent

of the first component to bind to the complementary nucleotide sequence of the capture reagent of the second component, and thereby form a pre-hybridized RNA-capture reagent complex comprising the target nucleotide sequence;

b) contacting the pre-hybridized RNA-capture reagent complex with a microarray having thereon a plurality of features each [containing] comprising a particular probe nucleotide sequence; and

c) incubating the pre-hybridized RNA-capture reagent complex on the microarray at a second temperature [and for a time sufficient] to hybridize the target nucleotide sequence of the pre-hybridized RNA-capture reagent complex to the complementary probe nucleotide sequence contained within the feature, wherein the presence of such hybridization results in [the emission of the detectable signal from the corresponding feature, and the absence thereof results in no emission of the detectable signal from the corresponding feature, thus generating] a detectable hybridization pattern for subsequent analysis.

19. (Once Amended) A method for determining the presence of a specific nucleotide sequence in an RNA reagent of a target sample, said method comprising the steps of:

a) contacting a first component including an RNA reagent extracted [directly] from a target sample, said RNA reagent having a target nucleotide sequence and a capture sequence with a microarray having thereon a plurality of features each [containing] comprising a particular probe nucleotide sequence;

b) incubating the RNA reagent and the complementary probe nucleotide sequences on the microarray at a first temperature [and for a time sufficient] to hybridize the target nucleotide

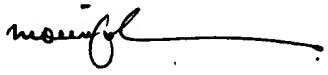
sequence of the RNA reagent to the complementary probe nucleotide sequence contained within the feature;

c) contacting a second component comprising a capture reagent [having at least one first arm containing] comprising a label capable of emitting a detectable signal and [at least one second arm having] comprising a nucleotide sequence complementary to the capture sequence of the RNA reagent of the first component; and

d) incubating the capture reagent and the capture sequence of the RNA reagent at a second temperature [and for a time sufficient] to induce the capture sequence of the RNA reagent of the first component to hybridize to the complementary nucleotide sequence of the capture reagent of the second component, wherein the presence of the hybridization results in [the emission of the detectable signal from the corresponding feature, and the absence thereof results in no emission of the detectable signal from the corresponding feature, thus generating] a detectable hybridization pattern for subsequent analysis.

Dated: January 3, 2003

Respectfully submitted,



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